Product Datasheet

BMAL1 Antibody - BSA Free
NB100-2288

Unit Size: 0.1 ml

Store at 4C for up to 3 months. For longer storage, aliquot and store at -20C.

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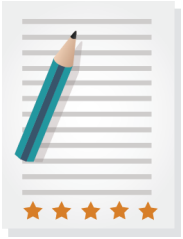


NB100-2288

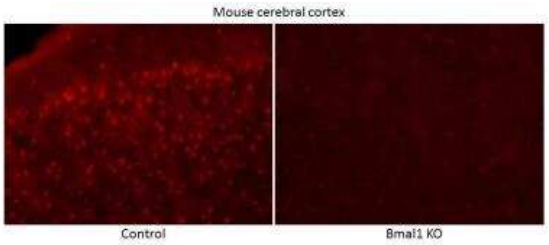
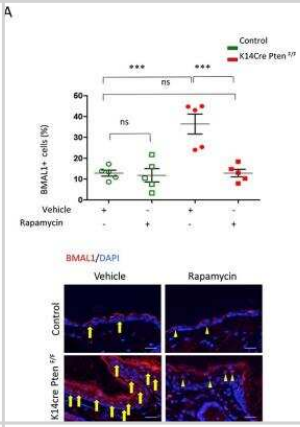
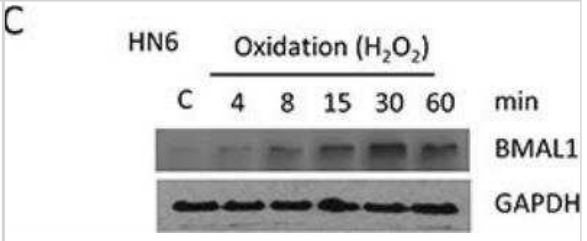
BMAL1 Antibody - BSA Free

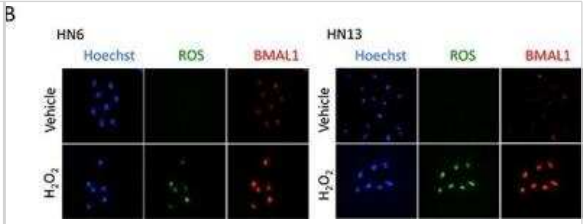
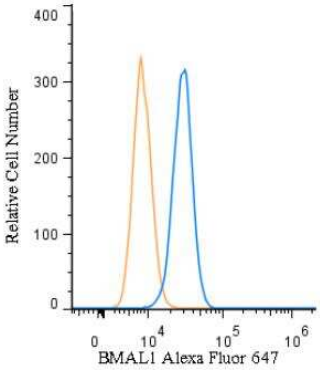
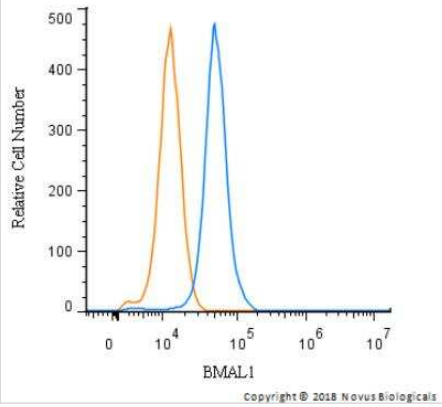
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C for up to 3 months. For longer storage, aliquot and store at -20C.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	70 kDa

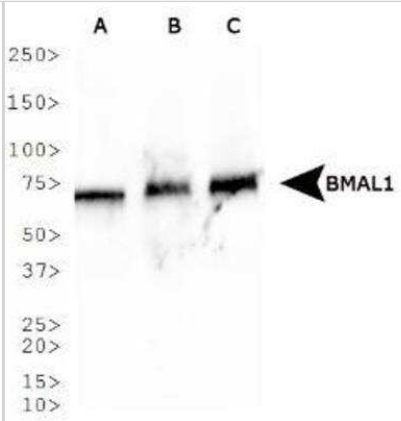
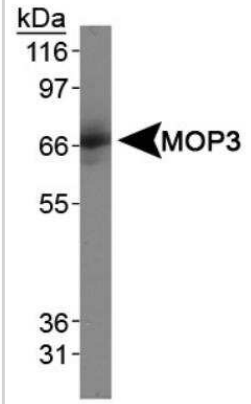
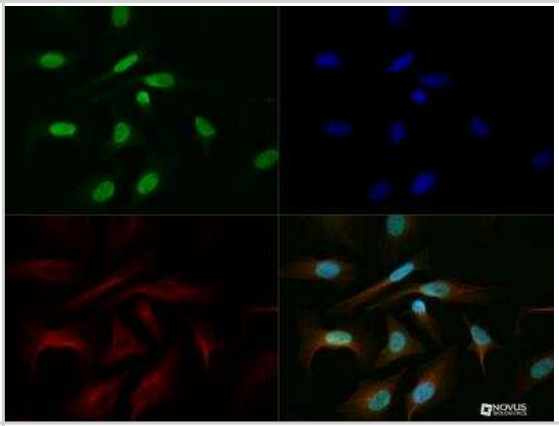
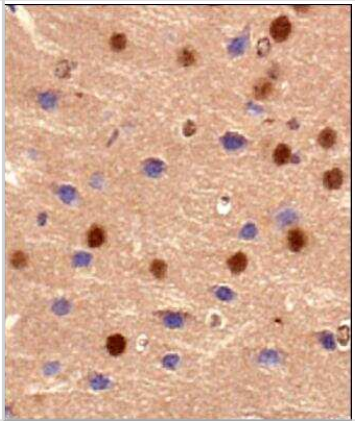
Product Description	
Host	Rabbit
Gene ID	406
Gene Symbol	BMAL1
Species	Human, Mouse, Rat, Amphibian, Primate
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID:32732906). Amphibian reactivity reported from a verified customer review.
Immunogen	Bacterially expressed human BMAL1 (amino acids 392-626). [UniProt# O00327].

Product Application Details	
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Chromatin Immunoprecipitation (ChIP), Knockout Validated
Recommended Dilutions	Western Blot 0.5 ug/mL - 2 ug/mL, Flow Cytometry, Immunohistochemistry 1:250. Use reported in scientific literature (PMID 33510438), Immunocytochemistry/ Immunofluorescence 1:100, Immunohistochemistry-Paraffin 1:250, Immunohistochemistry-Frozen reported in scientific literature (PMID 23736292), Flow (Intracellular), Chromatin Immunoprecipitation (ChIP), Knockout Validated
Application Notes	In ICC/IF, primarily nuclear staining was observed with weak cytoplasmic staining in MCF7 cells. In Western Blot, a band was observed ~70 kDa. In IHC-P, staining was observed in the nuclei of mouse brain tissue. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

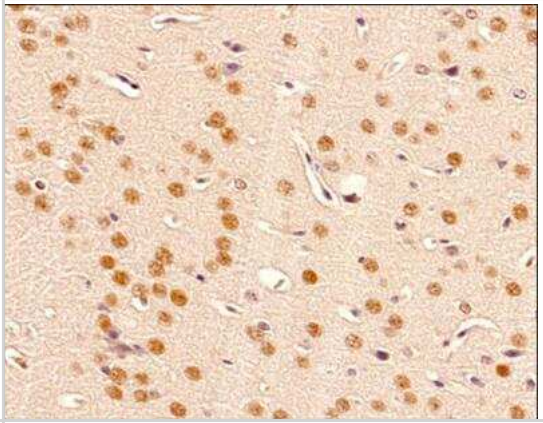
Images

Western Blot: BMAL1 Antibody [NB100-2288] - Oxidation causes accumulation of BMAL1. Head and neck cancer cells have different expression levels of the core clock protein BMAL1. Image collected and cropped by CiteAb from the following publication (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5173143/) licensed under a CC-BY license.	
Immunohistochemistry: BMAL1 Antibody [NB100-2288] - Analysis of BMAL1 in mouse cerebral cortex (left: wt, right: Bmal1 KO) using anti-BMAL1 antibody. Image from verified customer review.	
Immunohistochemistry: BMAL1 Antibody [NB100-2288] - Inhibition of mTOR signaling with Rapamycin rescues PTEN-driven accumulation of BMAL1A. Tissue samples and graphic show BMAL1 in K14cre PtenF/F mice and control (arrows). Note a significant amount of BMAL1 expression in PTEN mutant mice. K14cre PtenF/F mice and control littermates received Rapamycin or vehicle every other day for 15 days. Rapamycin treatment reduces the expression of nuclear BMAL1 in K14cre PtenF/F mice (arrowhead) (*** p<0.001) to similar levels found in control mice (ns: p>0.05). Scale bars -30um. Image collected and cropped by CiteAb from the following publication (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5173143/) licensed under a CC-BY license.	
Western Blot: BMAL1 Antibody [NB100-2288] - Oxidation causes accumulation of BMAL1. Western blot assay demonstrates time-dependent accumulation of BMAL1 upon oxidative stress in HNSCC cells (*p<0.05, **p<0.01). Image collected and cropped by CiteAb from the following publication (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5173143/) licensed under a CC-BY license.	

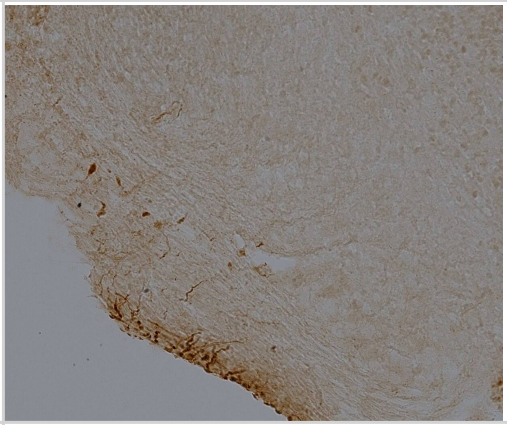
Immunocytochemistry/Immunofluorescence: BMAL1 Antibody [NB100-2288] - Oxidation causes accumulation of BMAL1. Immunofluorescence assay depicts accumulation of ROS (green channel) and BMAL1 (red channel) upon oxidation. Image collected and cropped by CiteAb from the following publication (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5173143/) licensed under a CC-BY license.	
Flow (Intracellular): BMAL1 Antibody [NB100-2288] - An intracellular stain was performed on HeLa cells with NB100-2288AF647(blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	
Immunohistochemistry: BMAL1 Antibody [NB100-2288] - CamiCre+; BMAL1fx/fx mice are forebrain/SCN-specific BMAL1 knockouts. (J) Immunohistochemistry for BMAL1 on SCN-containing coronal sections of Fx/Fx (n = 3), BKO (n = 3), and BMAL1-/- (KO, n = 1) mice sacrificed at ZT16. Captured with an 20x objective. F. CTX: frontal cortex, Cer: cerebellum. DOI:https://dx.doi.org/10.7554/eLife.04617.003Image collected and cropped by CiteAb from the following publication (https://elifesciences.org/articles/04617) licensed under a CC-BY license.	
Flow Cytometry: BMAL1 Antibody [NB100-2288] - An intracellular stain was performed on Jurkat cells with NB100-2288 and a matched isotype control. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody.	

Western Blot: BMAL1 Antibody [NB100-2288] - Analysis of BMAL1 in A) MCF7, B) NIH/3T3, C) PC12.	
Western Blot: BMAL1 Antibody [NB100-2288] - Analysis of MOP3 on 3T3/L1 cell lysates.	
Immunocytochemistry/Immunofluorescence: BMAL1 Antibody [NB100-2288] - BMAL1 antibody was tested in HeLa cells at a 1:200 dilution against Dylight 488 (Green). Alpha tubulin and nuclei were counterstained against DyLight 568 (Red) and DAPI (Blue), respectively.	
Immunohistochemistry: BMAL1 Antibody [NB100-2288] - Analysis of BMAL1 in mouse brain using DAB with hematoxylin counterstain.	

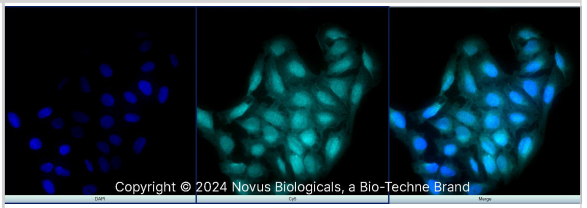
Immunohistochemistry-Paraffin: BMAL1 Antibody [NB100-2288] - Analysis of a FFPE tissue section of mouse brain using 1:200 dilution of rabbit anti-BMAL1 antibody. The staining was developed using HRP labeled anti-rabbit IgG secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin. This BMAL1 antibody generated a specific nuclear staining in most of the cells and a relatively weaker cytoplasmic signal was also observed.



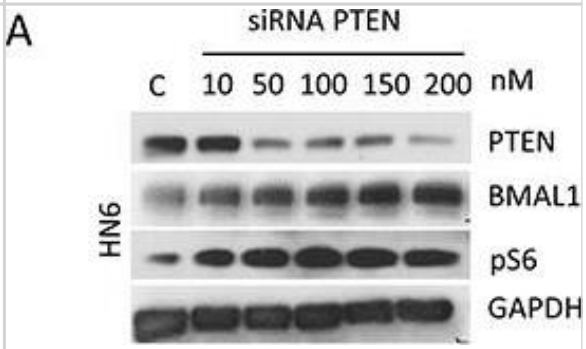
Immunohistochemistry-Frozen: Rabbit Polyclonal BMAL1 Antibody [NB100-2288] - IHC-fixed frozen in the brain of green tree frogs. DAB staining, 1:1000 dilution. Image from a verified customer review.



BMAL1 was detected in immersion fixed MCF7 human breast cancer cell line using Rabbit anti-BMAL1 Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NB100-2288AF647) (light blue) at 10 µg/mL overnight at 4C. Cells were stained counterstained with DAPI (blue). Cells were imaged using a 40X objective.



Immunocytochemistry/ Immunofluorescence: BMAL1 Antibody [NB100-2288] - Rapamycin reduces the accumulation of BMAL1 in Per2 knockout miceA. As shown in the left panel, tissue samples from Per2 knockout mice (mPER−/−) depict robust accumulation of nuclear BMAL1 (arrow) compared to control littermates (arrowhead)(*** p<0.001). Administration of Rapamycin reduces the accumulation of BMAL1 in the epidermis of mPer−/− mice (arrowhead) compared to mPer−/− mice receiving vehicle alone (** p<0.01) to levels comparable to wild-type mice receiving vehicle alone (ns: p>0.05). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27285754), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Western Blot: BMAL1 Antibody [NB100-2288] - Small interference RNA targeting Raptor & Rictor disrupts BMAL1 accumulation in HNSCCTargeted disruption of Raptor (A-B) & Rictor (C-D) using siRNA results in a dose-dependent downregulation of BMAL1 in HNSCC cells. E. Disruption of PTEN by protein oxidation causes activation of mTOR signaling, resulting in accumulation of BMAL1. Notably, inhibition of mTOR signaling, particularly mTORC1 & mTORC2, results in restoration of normal BMAL1 levels in the epidermis of mice & head & neck cancer cells. These results demonstrate a novel role for mTOR in regulating nuclear levels of the core clock gene BMAL1. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27285754), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: BMAL1 Antibody [NB100-2288] - Targeted disruption of PTEN in vitro & in vivo induces activation of pS6 & BMAL1A. & B. Targeted inhibition of PTEN using siRNA results in concentration-dependent inhibition of PTEN protein in HNSCC cells. HNSCC cells show accumulation of BMAL1 & pS6 in response to PTEN inhibition. C. Immunofluorescence assay to detect BMAL1 in PTEN conditional knockout mice (K14cre PtenF/F) & control littermates. Immunofluorescences & graphic show high accumulation of nuclear BMAL1 in K14cre PtenF/F mice compared to control mice (K14cre). Scale bars represent 50 µm. Black & white images depict cells positive for BMAL1 in K14cre PtenF/F mice (arrows) compared to few BMAL-positive cells in control mice (arrowhead). Scale bars represent 10 µm. (**p<0.01). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27285754), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
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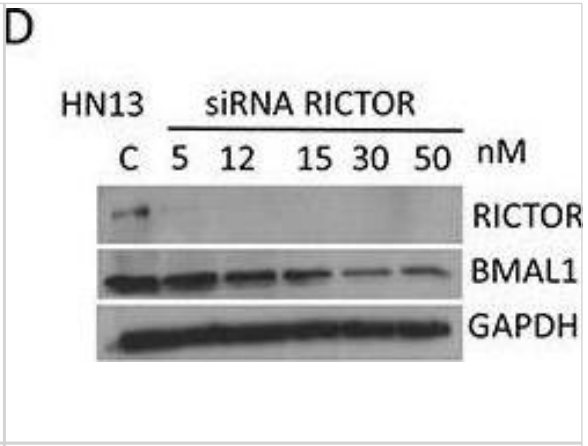
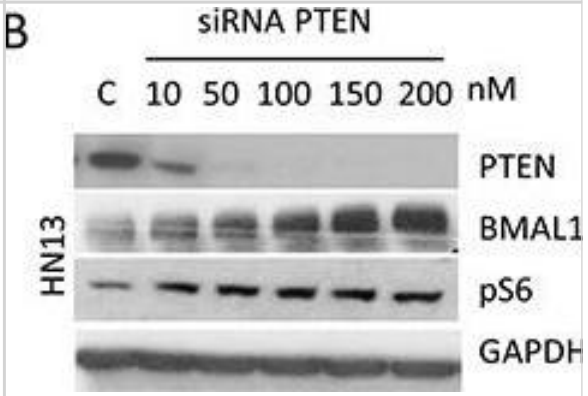
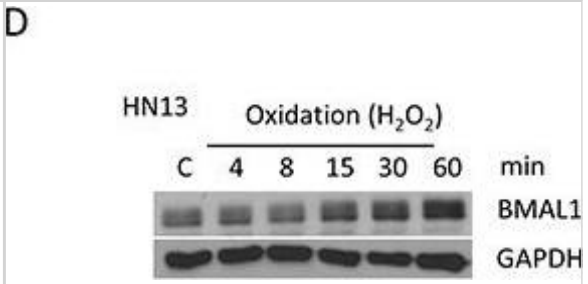
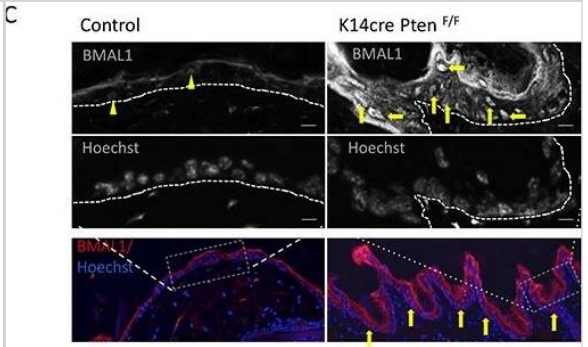
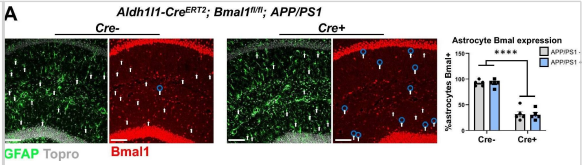
Western Blot: BMAL1 Antibody [NB100-2288] - Small interference RNA targeting Raptor & Rictor disrupts BMAL1 accumulation in HNSCCTargeted disruption of Raptor (A-B) & Rictor (C-D) using siRNA results in a dose-dependent downregulation of BMAL1 in HNSCC cells. E. Disruption of PTEN by protein oxidation causes activation of mTOR signaling, resulting in accumulation of BMAL1. Notably, inhibition of mTOR signaling, particularly mTORC1 & mTORC2, results in restoration of normal BMAL1 levels in the epidermis of mice & head & neck cancer cells. These results demonstrate a novel role for mTOR in regulating nuclear levels of the core clock gene BMAL1. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27285754), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: BMAL1 Antibody [NB100-2288] - Targeted disruption of PTEN in vitro & in vivo induces activation of pS6 & BMAL1A. & B. Targeted inhibition of PTEN using siRNA results in concentration-dependent inhibition of PTEN protein in HNSCC cells. HNSCC cells show accumulation of BMAL1 & pS6 in response to PTEN inhibition. C. Immunofluorescence assay to detect BMAL1 in PTEN conditional knockout mice (K14cre PtenF/F) & control littermates. Immunofluorescences & graphic show high accumulation of nuclear BMAL1 in K14cre PtenF/F mice compared to control mice (K14cre). Scale bars represent 50 µm. Black & white images depict cells positive for BMAL1 in K14cre PtenF/F mice (arrows) compared to few BMAL-positive cells in control mice (arrowhead). Scale bars represent 10 µm. (**p<0.01). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27285754), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: BMAL1 Antibody [NB100-2288] - Oxidation causes accumulation of BMAL1A. Head & neck cancer cells have different expression levels of the core clock protein BMAL1. B. Immunofluorescence assay depicts accumulation of ROS (green channel) & BMAL1 (red channel) upon oxidation. C. & D. Western blot assay demonstrates time-dependent accumulation of BMAL1 upon oxidative stress in HNSCC cells (*p<0.05, **p<0.01). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27285754), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunocytochemistry/ Immunofluorescence: BMAL1 Antibody [NB100-2288] - Targeted disruption of PTEN in vitro & in vivo induces activation of pS6 & BMAL1A. & B. Targeted inhibition of PTEN using siRNA results in concentration-dependent inhibition of PTEN protein in HNSCC cells. HNSCC cells show accumulation of BMAL1 & pS6 in response to PTEN inhibition. C. Immunofluorescence assay to detect BMAL1 in PTEN conditional knockout mice (K14cre PtenF/F) & control littermates. Immunofluorescences & graphic show high accumulation of nuclear BMAL1 in K14cre PtenF/F mice compared to control mice (K14cre). Scale bars represent 50 µm. Black & white images depict cells positive for BMAL1 in K14cre PtenF/F mice (arrows) compared to few BMAL-positive cells in control mice (arrowhead). Scale bars represent 10 µm. (**p<0.01). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27285754), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Immunohistochemistry: BMAL1 Antibody [NB100-2288] - Astrocyte Bmal1 regulates genes with conflicting effects on Aβ deposition. (A) Topro, GFAP, & BMAL1 staining in CA1 hippocampus of 4-month-old BMAL1 aKO; APP/PS1-21 mice & Cre- controls (scale bar = 100 µm). Arrows indicate astrocyte nuclei quantified as indicated by Topro nuclei surrounded by GFAP positivity. Blue circles indicate nuclei quantified as BMAL1 negative. Quantification of astrocytes counted as BMAL1- or BMAL1+ is shown on the right. n = 5 mice per group, **** = $p < 0.0001$ by two-way ANOVA with Sidak multiple comparisons test. (B) Heatmap of Fluidigm qPCR analysis of 20 genes involved in the circadian clock, glial activation, & Alzheimer's Disease in cortex from Aldh1l1-CreERT2; Bmal1fl/fl mice & Cre- controls with or without APP/PS1-21 or APPNL-G-F/wt (n = 6–8 mice per group). Two-way ANOVA analysis: c = significant main effect of Cre genotype, m = main effect of Aβ model, c*m = interaction effect of cre & Aβ model, - = no significance (all $p < 0.05$). (C) Individually plotted genes from A. * = $p < 0.05$, ** = $p < 0.005$, *** = $p < 0.0005$ by two-way ANOVA with Sidak multiple comparisons test. Panel B was made using GraphPad Prism version 9.2 (https://www.graphpad.com). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35110643), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Shen Y, Xu LR, Yan D, Zhou M et Al. BMAL1 modulates smooth muscle cells phenotypic switch towards fibroblast-like cells and stabilizes atherosclerotic plaques by upregulating YAP1 Biochim Biophys Acta Mol Basis Dis 2022-05-22 [PMID: 35598770]

Wagner PM, Fornasier SJ, Guido ME., et Al. Pharmacological Modulation of the Cytosolic Oscillator Affects Glioblastoma Cell Biology Cell Mol Neurobiol 2024-06-22 [PMID: 38907776]

Engeland WC, Massman L, Mishra S, Yoder JM et Al. The Adrenal Clock Prevents Aberrant Light-Induced Alterations in Circadian Glucocorticoid Rhythms Endocrinology 2018-10-16 [PMID: 30321360]

Engeland WC, Massman L, Miller L, Leng S et Al. Sex Differences in Adrenal Bmal1 Deletion-Induced Augmentation of Glucocorticoid Responses to Stress and ACTH in Mice Endocrinology 2019-08-10 [PMID: 31398249]

Kanan MF, Sheehan PW, Haines JN et Al. Neuronal deletion of the circadian clock gene Bmal1 induces cell-autonomous dopaminergic neurodegeneration JCI Insight 2024-01-23 [PMID: 38032732]

Bittman EL., , et Al. Anatomical Methods to Study the Suprachiasmatic Nucleus Methods Mol Biol 2023-12-27 [PMID: 35610428]

Gutierrez-Monreal MA, Wolff CA, Rijos EE et Al. Targeted Bmal1 restoration in muscle prolongs lifespan with systemic health effects in aging model JCI Insight 2024-10-01 [PMID: 39352748]

Liu Q, Xiong J, Kim DW et Al. An amygdalar oscillator coordinates cellular and behavioral rhythms Neuron 2024-09-18 [PMID: 39303704]

Rojo D, Dal Cengio L, Badner A et al. BMAL1 loss in oligodendroglia contributes to abnormal myelination and sleep Neuron 2023-08-25 [PMID: 37657440]

Lukasz P. Slomnicki, Scott A. Myers, Sujata Saraswat Ohri, Molly V. Parsh, Kariena R. Andres, Julia H. Chariker, Eric C. Rouchka, Scott R. Whittemore, Michal Hetman Improved locomotor recovery after contusive spinal cord injury in Bmal1 −/− mice is associated with protection of the blood spinal cord barrier Scientific Reports 2020-08-26 [PMID: 32848194]

Rachel Van Drunen, Yulin Dai, Haichao Wei, Baharan Fekry, Sina Noori, Samay Shivshankar, Rafael Bravo, Zhongming Zhao, Seung-Hee Yoo, Nicholas Justice, Jia Qian Wu, Qingchun Tong, Kristin Eckel-Mahan Cell-specific regulation of the circadian clock by BMAL1 in the paraventricular nucleus: Implications for regulation of systemic biological rhythms. Cell reports 2024-06-26 [PMID: 38935503]

Silke Kiessling, Lou Beaulieu-Laroche, Ian D. Blum, Dominic Landgraf, David K. Welsh, Kai-Florian Storch, Nathalie Labrecque, Nicolas Cermakian Enhancing circadian clock function in cancer cells inhibits tumor growth BMC Biology 2017-02-14 [PMID: 28196531]

More publications at http://www.novusbio.com/NB100-2288

Procedures

Western Blot protocol for BMAL1 Antibody (NB100-2288)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.

Immunocytochemistry/Immunofluorescence protocol for BMAL1 Antibody (NB100-2288)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Immunohistochemistry-Paraffin Protocol for BMAL1 Antibody (NB100-2288)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

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